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Evaluation of Phytochemicals and *In Vitro* Antioxidant and Anti-Inflammatory Properties of *Aframomum Daniellii* Rhizome Extract

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ABSTRACT

Aframomum daniellii is widely used in traditional medicine; however, scientific evidence supporting its bioactivity remains limited. This study quantified the phytochemical constituents and evaluated the in vitro antioxidant and anti-inflammatory activities of the ethanol extract of A. daniellii rhizome. Powdered rhizomes (250 g) were extracted with 80% (v/v) ethanol for 48 h and concentrated in vacuo at 40 ºC to obtain the ethanol extract of A. daniellii (EEAD). Total phenolics, flavonoids, saponins, alkaloids, vitamins C and E were quantified, while total antioxidant capacity, reducing power, hydrogen peroxide inhibition, DPPH radical scavenging, albumin denaturation inhibition, and membrane-stabilising assays were evaluated. EEAD contained flavonoids (46.79±1.44 mgRE/g), total phenolics (46.08 ± 0.45 mgTAE/g), saponins (18.63±0.17 mgQSE/g), alkaloids (13.06±2.41 mgSE/g), and vitamins C and E (4.63±0.04 mg/g and 0.21±0.04 mg/g, respectively). The extract scavenged hydrogen peroxide with an IC_{50} of 1208.06±6.34 µg/ml and exhibited DPPH radical scavenging activity with an IC_{50} of 85.57±0.11 µg/ml. Inhibition of albumin denaturation and membrane-stabilising activities were 28.63±0.71% and 63.09±1.08%, respectively. These findings demonstrate that EEAD is rich in bioactive phytochemicals and possesses notable antioxidant and anti-inflammatory properties, supporting its potential relevance in the management of oxidative stress- and inflammation-related conditions.

Keywords: *Medicinal Plants; Bioactive Constituents; Reactive Oxygen Species; Cell Membrane; Diseases; Ailments; Management.*

IJAMSR

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Introduction

Plants serve not only as sources of food for humans and animals but also play a crucial role in the management of ailments and diseases. Virtually every part of a plant is useful, either as food or as a source of raw materials for pharmaceutical and chemical production (Niazi and Monib 2024; Burchi and De Muro, 2016). Medicinal plants have shown considerable promise in the development of new therapeutic agents and in disease management. However, to substantiate their medicinal value, plants must undergo rigorous scientific evaluation (Davis and Choisy 2024).

The increasing demand for alternative and complementary medicine has intensified efforts to investigate medicinal plants for their potential health benefits. In many developing regions, medicinal plants remain the primary source of healthcare and are of immense importance to the pharmaceutical industry. These plants are also recognised as rich sources of natural antioxidants and phytochemicals (Karthishwaran *et al.*, 2018), thereby making research into antioxidant products particularly significant for both medical and food industries (Karthishwaran *et al.*, 2018).

Oxidative stress, arising from the excessive production of reactive oxygen species (ROS), is implicated in the pathogenesis of numerous diseases. This condition results from an imbalance between endogenous antioxidant defence mechanisms and ROS generation. Persistent oxidative imbalance can lead to cellular damage and subsequently trigger chronic and degenerative diseases (Sabahi *et al.*, 2018; Izuegbuna *et al.*, 2019). Antioxidants are chemical substances that protect body cells against damage caused by free radicals (Dluya *et al.*, 2017). Several studies on medicinal plants and vegetables have demonstrated that plant-derived constituents possess the capacity to protect biological systems against oxidative stress.

Although synthetic antioxidants have been widely employed with considerable success, their use has been associated with adverse effects, including carcinogenicity and other health concerns. Consequently, there has been a growing interest in the search for safer, natural antioxidant sources (Banothu *et al.*, 2017; Datta *et al.*, 2019). Prominent bioactive constituents found in plants include flavonoids, saponins, alkaloids, tannins, glycosides, and other phenolic compounds. Many plants contain appreciable amounts of antioxidants such as vitamins C and E, phenolics, alkaloids, saponins, flavonoids, and tannins, which are essential for scavenging excess free radicals in the body (Dluya *et al.*, 2017).

Aframomum daniellii (Hook. f.) K. Schum belongs to the family Zingiberaceae and is predominantly distributed across Central and West African countries. The plant typically grows under shaded conditions in plantations and riverine environments (Essien *et al.*, 2017). *A. daniellii* is a natural spice of the genus *Aframomum*, characterised by shiny olive-brown smooth seeds with a turpentine-like flavour, which are traditionally used for medicinal purposes. The plant possesses preservative properties and is rich in essential nutrients and minerals, including protein, calcium, magnesium, sodium, manganese, phosphorus, zinc, and copper.

Lawal *et al.* (2017) reported the use of *A. daniellii* essential oil in the inhibition of food spoilage organisms, yeasts, and mycotoxin-producing moulds. Further biological investigations have revealed that species of *Aframomum* exhibit diverse pharmacological activities, including antioxidant, antifungal, analgesic, antimicrobial, antiprotozoal, and aphrodisiac properties. In addition, the crude ethanol extract of *A. daniellii* seeds has been shown to possess significant antioxidative and flavour-enhancing properties (Afolabi and Adegoke, 2014).

IJAMSR

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This study was therefore designed to investigate the phytochemical composition, as well as the *in vitro* antioxidant and anti-inflammatory activities of *A. daniellii* rhizomes, with a view to exploring its potential application in the treatment and management of oxidative stress- and inflammation-related disorders.

Materials and Methods

Materials

Collection and Identification of Plant Material

Fresh rhizomes of *A. daniellii* (Hook. f) K. Schum were collected at Km 56 along the Ife–Ibadan Expressway, Ile-Ife, Osun State (04° 28′ 15″ E and 01° 29′ 54″ N). The plant material was identified and authenticated at the Forest Herbarium (FHI), Forestry Research Institute of Nigeria, Forestry Hill, Jericho, Ibadan, with voucher number FHI. 111056.

Reagents and Chemicals

All reagents used in this study were of analytical grade. Analar grade solvents and reagents, including sulphuric acid, phosphoric acid, n-hexane, butanol, diethyl ether, chloroform, ethyl acetate, ethanol, methanol, copper II sulphate ($CuSO_4$), vanillin, ascorbic acid, hydrogen peroxide (H_2O_2), trichloroacetic acid, bromocresol green, bovine serum albumin, and Quillaja saponin, were products of BDH Chemicals Ltd, Poole, England (now Merck Chemicals Ltd, Hull). Folin–Ciocalteu's phenol reagent and DPPH were purchased from Sigma-Aldrich, 3050 Spruce Street, St Louis, U.S.A. Aluminium chloride ($AlCl_3$), sodium hydrogen carbonate (NaHCO3), sodium hydroxide (NaOH), sodium nitrite (Na2NO3), sodium carbonate (Na_2CO_3), and sodium potassium tartrate were obtained from TLE Scientific. Disodium hydrogen phosphate (Na2HPO4) and sodium dihydrogen phosphate (NaH_2PO_4) were also used. Strychnine (alkaloid) and rutin (flavonoid) were purchased from Chadwell Heath, Essex, England. Sodium chloride (NaCl) was obtained from Oxford Laboratory, Mumbai, India.

Methods

Preparation of Ethanol Extract of *A. daniellii*

The ethanol extract of *A. daniellii* was prepared according to a modified method of Oyedapo and Amos (1997). The rhizomes were cut into small pieces, dried, and ground using a manual grinder. Briefly, powdered rhizomes (250 g) were soaked in 80% (v/v) ethanol (4 L) for 48 hr, with periodic agitation at regular intervals. After 48 hr, the mixture was filtered through two layers of cheesecloth. The residue was re-extracted with the same solvent (80% (v/v) ethanol) three additional times for 48 hr each, until the extracts became colourless, and were filtered as described above. The filtrates were pooled and centrifuged at 3,000 rpm for 10 min at room temperature using a Table Centrifuge (Model 90-2 Searchtech Instrument). The combined supernatant was evaporated to dryness at 40°C under reduced pressure using a Buchi Rotavapor RII Vacuum Pump, Model V-700 (Switzerland). The resulting residue, termed ethanol extract of *A. daniellii* (EEAD), was collected and stored in a desiccator until required for further analyses.

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

Estimation of Total Flavonoids Content of Ethanolic Extract of *A. daniellii*

Total flavonoid content of EEAD was determined according to Singh *et al.* (2010). Reaction mixtures consisted of 0.5 ml of extract (1 mg/ml), distilled water (1.5 ml), 5 % (w/v) $NaNO_2$ (0.3 ml), 10 % (w/v) $AlCl_3$ (0.3 ml), and 4 % (w/v) NaOH (2.0 ml). The mixture was incubated at room temperature for 10 min, after which absorbance was measured at 500 nm against the reagent blank. A standard calibration curve was prepared by pipetting varying volumes (0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml) of rutin (w/v; 1 mg/ml) in triplicate into clean test tubes. The reaction mixture was made up to 2 ml with distilled water and treated as described above. Flavonoid concentration was extrapolated from the standard curve and expressed as milligram rutin equivalent per g of extract (mg RE/g extract).

Estimation of Total Phenolics Content of EEAD

Total phenolic content of EEAD was determined according to Singleton *et al.* (1999) as slightly modified by Akinlalu *et al.* (2016). The reaction mixture contained 1.0 ml of extract (1 mg/ml), 10 % (w/v) $NaHCO_3$ (1.5 ml), and Folin–Ciocalteu's phenol reagent (1.5 ml, 1:10). The mixture was incubated in the dark for 11/2 hr and absorbance measured at 725 nm against the reagent blank. A standard calibration curve was prepared using tannic acid (w/v; 20 µg/ml) by pipetting varying volumes (0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml) in triplicate into clean test tubes. The reaction mixtures were made up to 1 ml with distilled water and treated as described above. Phenolic concentration was extrapolated from the curve and expressed as milligram tannic acid equivalent per g of extract (mg TAE/g extract).

Estimation of Total Saponins Content of EEAD

Saponin concentration was estimated using a procedure based on Akinpelu *et al.* (2012), as slightly modified by Morakinyo *et al.* (2018). Briefly, EEAD (5 g) was successively washed with n-hexane, chloroform, ethyl acetate, followed by addition of 50 ml of 20% (v/v) methanol. The mixture was extracted three times with n-butanol (100 ml x 3) and evaporated to dryness. The residue was dissolved in 50% (v/v) methanol, and diethyl ether (20 ml x 3) was added to precipitate crude saponin. The crude saponin was dried in an oven (55 °C) and used for spectrophotometric estimation.

Total saponin concentration was quantified using the vanillin–sulphuric acid reaction method (Hiai *et al.*, 1976) as modified by Makkar *et al.* (2007). The isolated saponin was dissolved in 80% (v/v) methanol to 1 mg/ml. From this solution, 0.25 ml was dispensed in triplicate into test tubes, followed by 0.25 ml of vanillin reagent (8% (w/v) in ethanol) and 2.5 ml of 72% (v/v) sulphuric acid. The mixture was homogenised and incubated at 60 ℃ in a water bath for 10 min. Absorbance was measured at 544 nm against a reagent blank containing 80% (v/v) methanol in place of crude saponin. A standard calibration curve was prepared using Quillaja saponin (w/v; 125 µg/ml) by pipetting 0.0, 50, 100, 150, 200, and 250 µl in triplicate into clean test tubes, making up to 250 µl with distilled water and treating as above. Saponin concentration in EEAD was extrapolated and expressed as milligram Quillaja saponin equivalent per g of extract (mg QSE/g extract).

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

Estimation of Total Alkaloids Content of Ethanol Extract of *A. daniellii*

Total alkaloid concentration of EEAD was determined using the bromocresol green reaction method (Shamsa *et al.*, 2008), with strychnine (1 mg/ml) as the standard alkaloid. EEAD (0.01 g; 1 mg/ml) was dissolved in 2 M HCl (10 ml) and filtered. The filtrate (1 ml) was dispensed in triplicate into test tubes, followed by addition of 50 mM phosphate buffer (pH 7.0; 5 ml). The mixture was transferred to a separating funnel and 5 ml of bromocresol green (BCG) solution (69.8 mg bromocresol green + 3 ml 2M NaOH + 5ml distilled water and was made up to a litre) was added and shaken. The yellow BCG–alkaloid complex was extracted with chloroform (1 ml x 5), collected into test tubes, and absorbance measured at 470 nm against the reagent blank. A standard calibration curve was prepared by pipetting strychnine (1 mg/ml) 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 m1 in triplicate, making up to 1 ml with 2 M HCl, and proceeding as above. Alkaloid concentration in EEAD was extrapolated and expressed as milligram strychnine equivalent per g of extract (mg SE/g extract).

Evaluation of Total Antioxidant Capacity (TAC) by Phosphomolybdenum Reaction

Total antioxidant capacity of EEAD was assessed by the phosphomolybdenum method (Prieto *et al.*, 1999) with slight modification. EEAD (1 mg/ml; 0.3 ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was incubated at 100 oC for 90 min and allowed to cool, after which absorbance was read at 695 nm against the blank. Methanol (0.3 ml) was used as the blank in place of extract/standard. A standard calibration curve was prepared using ascorbic acid (0 - 100 µg/ml). Antioxidant capacity was expressed as milligram equivalent of ascorbic acid per gram of extract.

Estimation of Vitamin C Concentration

Vitamin C concentration in EEAD was quantified using the Folin–Ciocalteu phenol reagent method as reported by Fajobi *et al.* (2017). Briefly, 0.5 ml of EEAD was de-proteinated with 2 % (v/v) orthophosphoric acid for 10 min at room temperature with vigorous stirring and centrifuged at 3500 for 10 min. The supernatant (0.5 ml) was mixed with 1.5 ml of 10% (v/v) acetic acid and 0.5 ml of Folin–Ciocalteu reagent (1:10 dilution). The mixture was incubated at room temperature for 10 min and absorbance read at 760 nm against the reagent blank. Vitamin C concentration was obtained using a standard curve prepared with ascorbic acid (0 – 20 µg/ml).

Estimation of Vitamin E Concentration

Total vitamin E in EEAD was quantified based on method described by Santhosh *et al.* (2013). The method is based on reduction of ferric to ferrous ions by vitamin E, followed by formation of a red-coloured complex with 2, 2-bipyridyl. For extraction, EEAD (0.5 g) was dissolved in ethanol (20 ml), incubated at 85°C, cooled, and filtered. The filtrate was extracted with heptane (10 ml x 5), and 1.25% (w/v) $NaSO_4$ (2 ml) was added, stirred, and shaken for 2 min. The extracted vitamin E (1 ml) was mixed with 1 ml 2,2-bipyridyl reagent (0.12 % (w/v)) and 1 ml ferric chloride (0.12 % (w/v)). Absorbance was read at 492 nm against the reagent blank. A standard calibration curve was prepared using vitamin E (0 – 20 µg/ml). Vitamin E content was expressed as milligram per gram of extract.

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

Evaluation of Ferric-Reducing Antioxidant Power (FRAP)

Reducing power of EEAD was evaluated based on Oyaizu (1986), as reported by Komolafe *et al.* (2024). EEAD (1 ml; 0 – 400 µg/ml) was mixed with 0.2 M phosphate buffer (pH 6.6; 2.5 ml) and 1% potassium ferricyanide [$K_3Fe(CN)6$] (2.5 ml). The mixture was incubated at 50 ºC for 20 min. Trichloroacetic acid (TCA) (2.5 ml; 10%, w/v) was added, and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.1% (w/v) $FeCl_3$ (2.5 ml). Absorbance was measured at 700 nm. Ascorbic acid (0–400 µg/ml) served as the standard using the same procedure.

Evaluation of Hydrogen Peroxide Radical Scavenging Activity

Hydrogen peroxide scavenging activity of EEAD was determined by the method of Klein *et al.* (1991), as reported by Archana and Vijayalakshmi (2017). The reaction mixture contained 1 ml of extract at different concentrations (0 – 1000 µg/ml), 0.5 ml of phosphate buffer (20 mM; pH 7.4), and 0.4 ml of 2 mM hydrogen peroxide. The mixture was incubated at room temperature for 5 min. Thereafter, 2 ml of dichromate–acetic acid reagent (5 %, w/v) was added and absorbance measured at 570 nm. Ascorbic acid (0 – 1000 µg/ml) served as the standard using the same procedure. The percentage inhibition of hydrogen peroxide was calculated using the expression:

$$\text{Percentage Inhibition of } H_2O_2 = \frac{Abs_{test} - Abs_{control}}{Abs_{test}} \times 100$$

Where, Abstest = absorbance of extract/standard and Abscontrol = absorbance of standard

Evaluation of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity

DPPH radical scavenging activity of EEAD was determined according to Blois (1985), as reported by Komolafe *et al.* (2021). The reaction mixture contained 1 ml of extract at varying concentrations (0 – 50 µg/ml) and 0.3 mM DPPH in methanol (1 ml). The solution was mixed and incubated in the dark for 30 min. Absorbance was measured at 517 nm against methanol as blank. The control contained 1 ml methanol in place of extract. Ascorbic acid served as the standard using the same procedure. Percentage DPPH radical scavenging activity was calculated using the expression:

$$\text{Percentage Scavenging Activity} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \text{ x } 100$$

Where, $Abs_{control}$ = absorbance of control and Abs_{sample} = absorbance of sample/standard

Evaluation of Inhibition of Albumin Denaturation Activity

Inhibition of albumin denaturation by EEAD was estimated according to Mizushima and Kobayashi (1968), with slight modification as reported by Aina and Oyedapo (2013). Bovine serum albumin (0.5 ml; 1.5 mg/ml) was added to extracts of varying concentrations (0 - 1000 µg/ml), followed by incubation at 37 ℃ for 20 min. The reaction mixture was further incubated at 57 ℃ for 3 min. The mixture was allowed to cool, after which 2.5 ml of 0.5 M phosphate buffer (pH 6.3) was added. From the reaction mixture, 1 ml was taken and mixed with 1 ml alkaline copper reagent and 1 ml Folin–Ciocalteau (10%). The mixture was incubated at 55 ℃ for 10 min, cooled, and absorbance measured

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

at 650 nm against the reagent blank. Aspirin served as the standard using the same procedure. Quantity of protein left and percentage inhibition were calculated using the expressions:

Quantity of protein left = $\frac{(Abs_{test} - Abs_{control})}{Abs_{test}}$

Percentage of inhibition = $\frac{Quantity\ of\ protein\ left}{Total\ protein}\ x\ 100$

Where, Abs_{test} = absorbance of extract/standard and $Abs_{control}$ = absorbance of standard

Evaluation of Membrane Stabilizing Activity

Membrane stabilising activity of EEAD was determined based on the method described by Aina and Oyedapo (2013). The assay mixture consisted of hyposaline (0.42% (w/v) NaCl; 1 ml), 0.1 M phosphate buffer (pH 7.4; 0.5 ml), varying concentrations of extracts/standard (0 – 500 µg/ml), and 0.5 ml of 2% (v/v) erythrocyte suspension. Drug control and blood control were prepared without erythrocytes and without drug, respectively, following the same procedure. Reaction mixtures were incubated at 56℃ for 30 min and absorbance measured at 560 nm against the reagent blank. Ibuprofen and diclofenac (0 – 500 µg/ml) were used as standard anti-inflammatory drugs. Percentage membrane stability was calculated using the expression:

Percentage membrane stability = [100 –{$\frac{Abs_{drug\ test} - Abs_{drug\ control}}{Abs_{blood\ control}}$}] X 100

Blood control will represent 100% lysis.

Where $Abs_{drug\ test}$ = absorbance of drug test, $Abs_{drug\ control}$ = absorbance of drug control and $Abs_{blood\ control}$ = absorbance of blood control.

Statistical Analysis

Results were expressed as mean ± standard error of mean of three parallel measurements. Linear regression analysis was used to determine IC50 values. Ms Excel and Graph Pad Prism version 5.0 were used for data analysis

Results and Discussion

Secondary plant metabolites are compounds that are not directly involved in plant growth, development, or reproduction but play important roles in survival and defense within their environment. Presence of alkaloids and flavonoids in the seeds of *A. daniellii* had been reported. In the present study, extraction of 250 g of *A. daniellii* rhizomes with 80% (v/v) ethanol yielded 20.92 g of crude extract, representing 8.37% of the starting material. This yield suggests efficient recovery of ethanol-soluble phytochemicals from the rhizomes.

Phenolic compounds are well recognised for their strong antioxidant capacity, which is attributed to their ability to scavenge free radicals, donate hydrogen atoms or electrons, and chelate metal cations. The antioxidant effectiveness of phenolics largely depends on their molecular structure, particularly the number and position of hydroxyl groups and the nature of substitutions on the aromatic rings (Platzer *et al.*, 2022). Flavonoids are another important class of phytochemicals with wide-ranging

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

biochemical properties such as anti-inflammatory, cardioprotective, antidiabetic, antiviral, antibacterial, anticancer, and anti-ageing activities (Hasnat *et al.*, 2024). Their mechanisms of action include suppression of reactive oxygen species (ROS) formation through enzyme inhibition or chelation of trace elements involved in free radical generation, direct scavenging of ROS, and up-regulation of endogenous antioxidant defence systems (Banothu *et al.*, 2017).

Saponins are structurally diverse compounds that exhibit a broad spectrum of biological activities, including anti-inflammatory, antifungal, anticancer, antitumour, cytotoxic, insecticidal, haemolytic, antibacterial, antiviral, and immunomodulatory properties. They are also known to display antioxidant activity (Izuegbuna *et al.*, 2019).

Alkaloids are widely exploited as pharmaceuticals, stimulants, narcotics, and poisons due to their potent biological activities. They have been reported to exhibit anti-inflammatory, antiplasmodial, insecticidal, and hepatoprotective effects (Debnath *et al.*, 2018).

In the present study, four major groups of secondary metabolites—total phenolics, flavonoids, saponins, and alkaloids—were quantified in the ethanol extract of *A. daniellii* rhizomes. As presented in Table 1, the contents of total phenolics, flavonoids, saponins, and alkaloids in EEAD were 46.08 ± 0.45 mgTAE/g, 46.79 ± 1.44 mgRE/g, 18.63 ± 0.17 mgQSE/g, and 13.06 ± 2.41 mgSE/g, respectively. These results indicate that EEAD contains appreciable amounts of bioactive phytochemicals, which may collectively contribute to its observed antioxidant and anti-inflammatory activities.

The antioxidant potential of EEAD was further assessed through its ability to reduce molybdenum (Mo^{6+}) to Mo^{5+} (Izuegbuna *et al.*, 2019). The total antioxidant capacity of the extract was 331.71 ± 12.90 mg ascorbic acid equivalent per gram of extract (Table 1). This activity may be largely attributed to the phenolic constituents present in the extract, as previous studies have reported a strong positive correlation between total phenolic content and antioxidant activity (Tananaki *et al.*, 2024). The result confirms the antioxidant potential of EEAD and its capacity to neutralise free radicals.

Vitamins play essential roles in maintaining cellular homeostasis by neutralising ROS and acting as antioxidant adjuvants (Izuegbuna *et al.*, 2019). Vitamins C and E are among the most extensively studied dietary antioxidants. Vitamin C neutralises ROS in the aqueous phase before the initiation of lipid peroxidation and is regarded as the most important water-soluble antioxidant in extracellular fluids (Anosike *et al.*, 2018; Izuegbuna *et al.*, 2019). Vitamin E, a major lipid-soluble antioxidant, protects membrane fatty acids from lipid peroxidation and acts as a chain-breaking antioxidant within cell membranes (Anosike *et al.*, 2018). In this study, vitamin C and E contents of EEAD were 4.63 ± 0.04 mg/g and 0.21 ± 0.04 mg/g, respectively (Table 1). These findings suggest that *A. daniellii* rhizome is a rich source of antioxidant vitamins, which may contribute to membrane protection, including red blood cell membrane stability, and protection against microbial infections (Anosike *et al.*, 2018). The results are consistent with the findings of Anosike *et al.* (2018), who reported appreciable amounts of vitamins C and E in the methanol extract of *Mucuna pruriens* leaves. Reactive oxygen species (ROS) induce oxidative damage that can lead to cell membrane

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

disintegration, DNA mutation, and protein damage, thereby initiating or propagating diseases such as cancer, cardiovascular disorders, and liver injury (An *et al.*, 2024).

The reducing power of EEAD was evaluated based on its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Reducing activity is associated with the presence of compounds capable of donating electrons or hydrogen atoms, thereby terminating free radical chain reactions (Anosike *et al.*, 2019). An increase in absorbance indicates increased reducing power. The reducing power of EEAD rhizome increased with concentration, showed a decline at 300 µg/ml, and subsequently increased at 400 µg/ml (Figure 1). Although the reducing power of the standard was higher than that of EEAD, the extract demonstrated appreciable reducing ability. This suggests that EEAD can act as an electron donor and convert free radicals into more stable products (Irshad *et al.*, 2012; Anosike *et al.*, 2019). Similar antioxidant potentials have been reported for various plant extracts exhibiting high FRAP values (Lalhminghlui and Jagetia, 2018). The reduction of Fe^{3+} to Fe^{2+} further confirms the electron-donating ability of EEAD (Arika *et al.*, 2019).

Hydrogen peroxide is a reactive oxygen species capable of inactivating enzymes through oxidation of essential thiol (–SH) groups and interacting with redox-active transition metals such as Fe^{2+} and Cu^{+}, thereby penetrating biological membranes (Sabahi *et al.*, 2018; Arika *et al.*, 2019). In this study, EEAD scavenged hydrogen peroxide in a concentration-dependent manner (Figure 2). Ascorbic acid exhibited the highest inhibition (79.85 ± 0.08%) at 1000 µg/ml, while EEAD showed a maximum inhibition of 40.36 ± 0.55% at the same concentration. The IC_{50} values were 382.65 ± 1.78 µg/ml for the standard and 1208.06 ± 6.34 µg/ml for the extract. These results indicate that EEAD possesses hydrogen peroxide scavenging activity, which may be attributed to its phenolic constituents (Arika *et al.*, 2019).

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay is one of the most widely used and reliable methods for evaluating antioxidant activity, based on the reduction of DPPH by hydrogen-donating antioxidants (Irshad *et al.*, 2012). As shown in Figure 3, the highest DPPH radical scavenging activity of EEAD was 31.16 ± 1.84% at 40 µg/ml, whereas ascorbic acid exhibited 87.87 ± 0.24% inhibition at 50 µg/ml. The IC_{50} values of EEAD and the standard were 85.57 ± 0.11 µg/ml and 19.74 ± 0.58 µg/ml, respectively, indicating stronger activity of the standard. Nevertheless, EEAD demonstrated notable radical scavenging ability, suggesting its capacity to donate protons and neutralise free radicals. This activity may be associated with the presence of phenolic compounds in the extract (Banothu *et al.*, 2017; Arika *et al.*, 2019).

Inflammation is a primary physiological defense response to various stimuli. Protein denaturation, induced by external stress or chemical agents, leads to loss of secondary, tertiary, and quaternary protein structures and has been linked to inflammatory diseases such as arthritis, cancer, and stroke (Ruiz-Ruiz *et al.*, 2017). Inhibition of protein denaturation is therefore considered an indicator of anti-inflammatory potential (Osman *et al.*, 2016). The highest inhibition of albumin denaturation observed for the standard (aspirin) and EEAD was 30.85 ± 0.76% and 28.63 ± 0.71%, respectively, at 1000 µg/ml (Figure 4). Although neither achieved 50% inhibition, EEAD compared favourably with the standard, indicating moderate anti-inflammatory potential.

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

Erythrocytes have been widely used as a model to study membrane stabilising effects of drugs and plant extracts (Podsiedlik *et al.*, 2020). Several phytochemicals, including saponins and flavonoids, have been reported to exhibit anti-inflammatory effects through membrane stabilisation (Chaity *et al.*, 2016; Oyine, 2018). As shown in Figure 5, EEAD demonstrated increased membrane stabilising activity from 300 µg/ml upwards, comparable to ibuprofen and diclofenac. At 500 µg/ml, EEAD exhibited the highest stabilising activity (63.09 ± 1.08%) compared with ibuprofen (50.09 ± 1.00%) and diclofenac (51.57 ± 0.83%). The membrane stabilising effects were concentration-dependent and monophasic. The observed activity may be attributed to the presence of flavonoids, phenolics, and saponins, either individually or through synergistic interactions (Oyine, 2018).

Conclusion

This study demonstrated that the ethanol extract of *A. daniellii* rhizome contains substantial quantities of bioactive phytochemicals and exhibits significant antioxidant, free radical scavenging, and anti-inflammatory activities. These properties support the potential usefulness of *A. daniellii* rhizome in the prevention and management of oxidative stress- and inflammation-related diseases.

Acknowledgement

Not Applicable

Table I. Phytochemical Composition and Antioxidant Potentials of Ethanol Extract of *A. daniellii* Rhizome (EEAD).

EEAD	Concentrations
Total Flavonoids (mgRE/g)	46.79 ± 1.44
Total Phenolics (mgTAE/g)	46.08 ± 0.45
Total Saponins (mgQSE/g)	18.63 ± 0.17
Total Alkaloids (mgSE/g)	13.06 ± 2.41
Total Antioxidant Capacity (mgAAE/g)	331.71 ± 12.90
Vitamin C (mg/g)	4.63 ± 0.04
Vitamin E (mg/g)	0.21 ± 0.04

Values are expressed as mean ± SEM (n = 3). Total flavonoids, phenolics, saponins, alkaloids, and total antioxidant capacity are expressed as rutin equivalent (RE), tannic acid equivalent (TAE), Quillaja saponin equivalent (QSE), strychnine equivalent (SE), and ascorbic acid equivalent (AAE), respectively.

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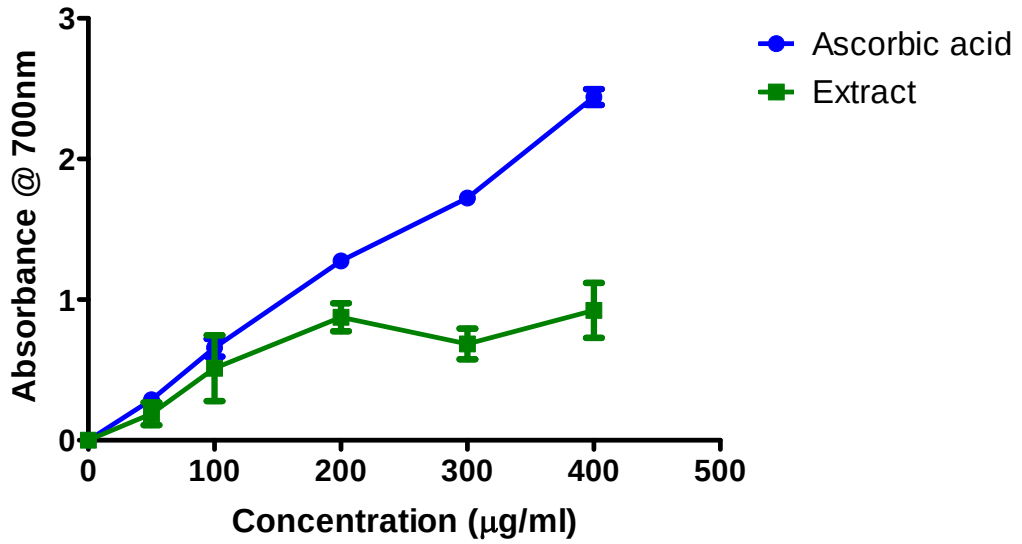

Figure I. Ferric-reducing antioxidant power (FRAP) activity of ethanol extract of A. daniellii rhizome (EEAD). Values represent mean ± SEM (n = 3).

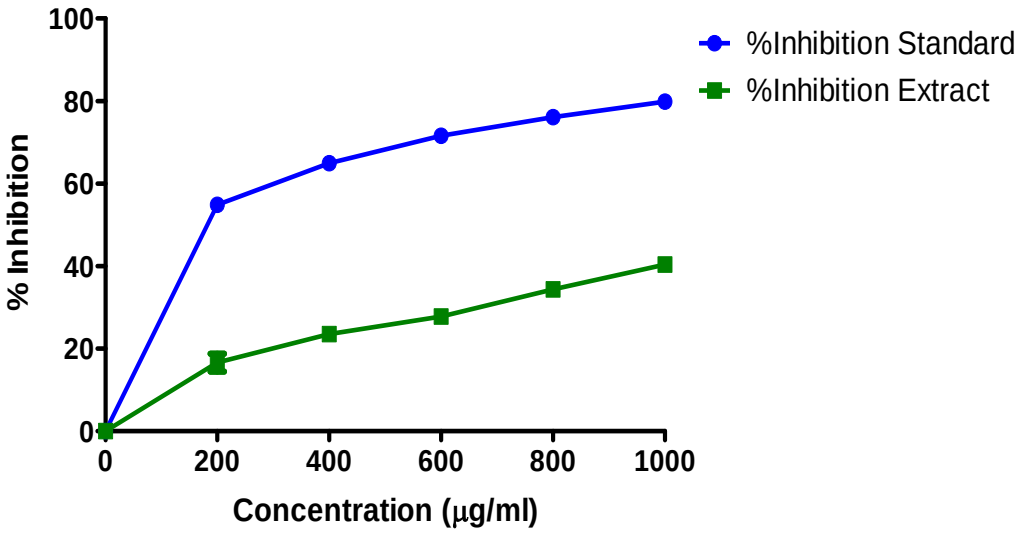

Figure II. Hydrogen peroxide radical scavenging activity of ethanol extract of A. daniellii rhizome (EEAD). Values represent mean ± SEM (n = 3).

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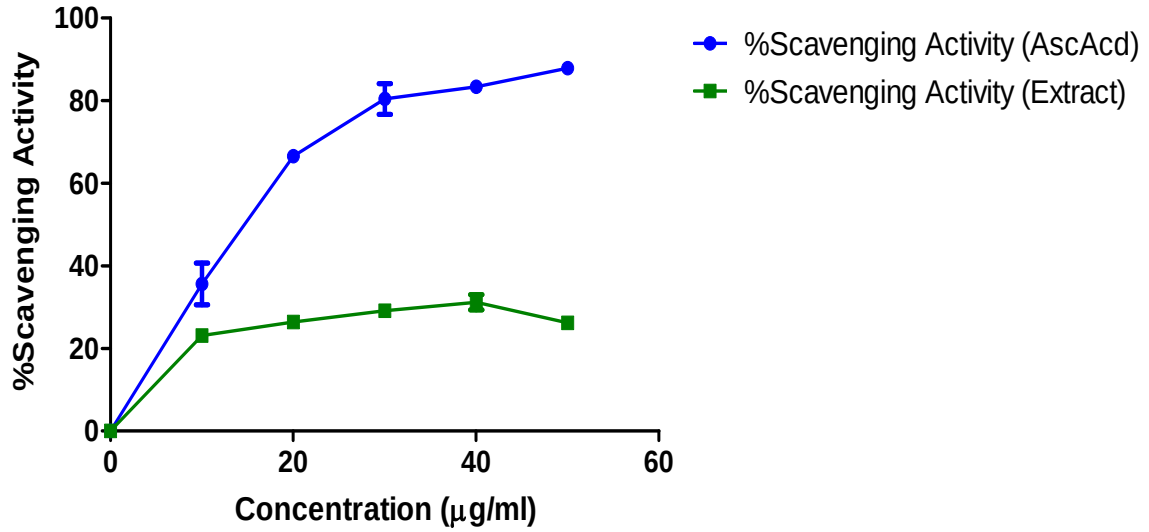

Figure III. DPPH radical scavenging activity of ethanol extract of A. daniellii rhizome (EEAD). Values represent mean ± SEM (n = 3).

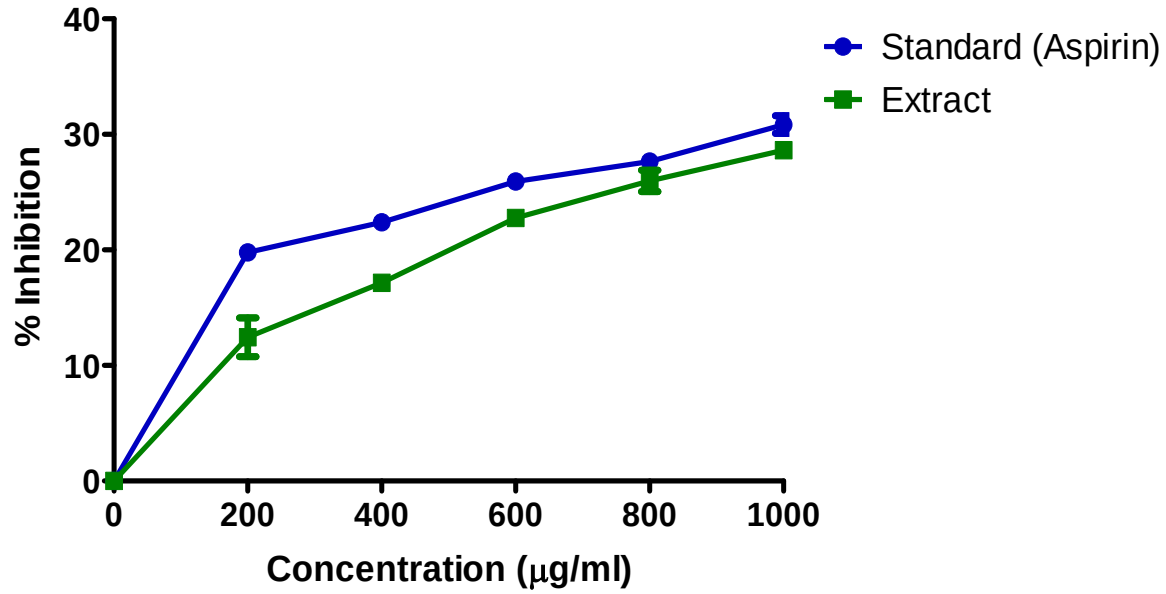

Figure IV. Inhibition of albumin denaturation by ethanol extract of A. daniellii rhizome (EEAD) compared with aspirin. Values represent mean ± SEM (n = 3).

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International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

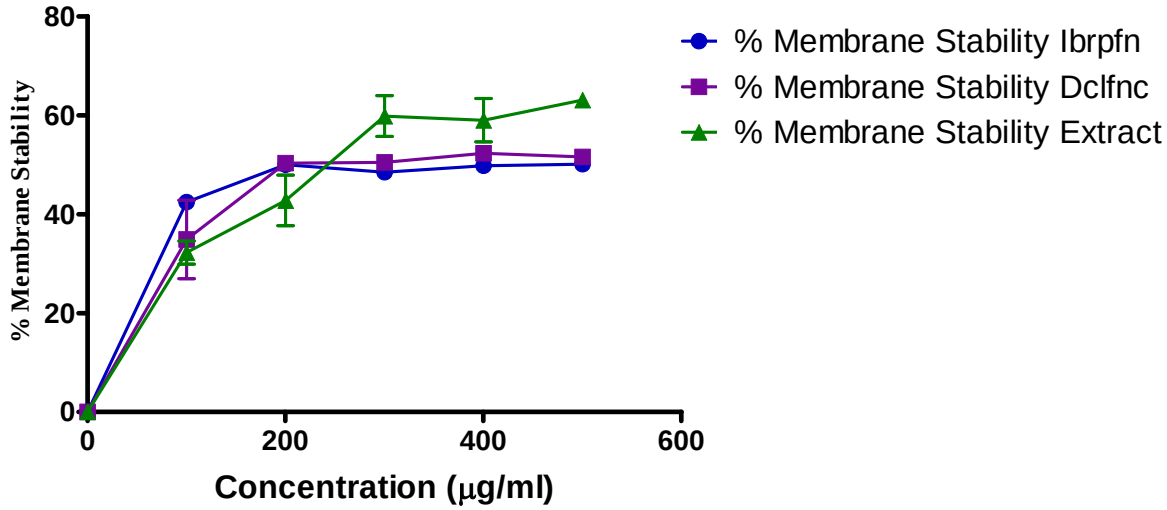

Figure V. Membrane stabilising activity of ethanol extract of A. daniellii rhizome (EEAD) compared with ibuprofen and diclofenac. Values represent mean ± SEM (n = 3).

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